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REGULATION OF GENE EXPRESSION

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For all living cells, regulation of gene expression by extracellular signals is a fundamental mechanism of development, homeostasis, and adaptation to the environment. Indeed, the ultimate step in many signal transduction pathways is the modification of transcription factors that can alter the expression of specific genes. Thus, neurotransmitters, growth factors, and drugs are all capable of altering the patterns of gene expression in a cell. Such transcriptional regulation plays many important roles in nervous system functioning, including the formation of long-term memories. For many drugs, which require prolonged administration for their clinical effects (e.g., antidepressants, antipsychotics), the altered pattern of gene expression represents therapeutic adaptations to the initial acute action of the drug.

Mechanisms that underlie the control of gene expression are becoming increasingly well understood. Every conceivable step in the process is subject to dynamic regulation in the cell. This includes structural changes in the chromatin to make a particular gene accessible for transcription, transcription of DNA into RNA, splicing of RNA into mRNA, editing and other covalent modifications of the mRNA, translation of mRNA into protein, and, finally, post-translational modification of the protein into its mature, functional form.

Molecular details of each of these regulatory steps are becoming increasingly available. In this chapter, we focus on the regulation of gene expression by transcription factors because their role in mediating the ability of extracellular signals to alter gene expression remains the best characterized.

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OVERVIEW OF TRANSCRIPTIONAL CONTROL MECHANISMS

Regulation of Gene Expression by the Structure of Chromatin

In eukaryotic cells, DNA is contained within a discrete organelle called the nucleus, which is the site of DNA replication and transcription. Within the nucleus, chromosomes—which are extremely long molecules of DNA—are wrapped around histone proteins to form nucleosomes, the major subunits of chromatin (1–3). To fit within the nucleus, much of the DNA is tightly packed into a ''coiled coil.'' Compared with transcriptionally quiescent regions, actively transcribed regions of DNA may be more than 1,000-fold further extended. Chromatin does not just serve a structural role, however; in eukaryotes, chromatin plays a critical role in transcriptional regulation. Chromatin can inhibit access of transcription factors to the DNA and can thereby repress gene expression. In eukaryotic organisms, with their very large number of genes (approximately 40×10^3 in mammals), this means that the ground state of gene expression is for genes to be turned off. Activation of gene expression requires that cells alleviate nucleosome-mediated repression of an appropriate subset of genes. This is accomplished by means of activator proteins that modify chromatin structure. The activation process, which involves transcription factors, along with histones and cofactors, displaces or remodels chromatin, and opens up regions of the DNA, including the core promoters (see later) of genes, for the binding of regulatory proteins.

Transcription occurs when particular activator proteins displace nucleosomes. This permits a complex of proteins (described later) called *general transcription factors,* to bind DNA at a particular type of element, called a *core promoter,* and to recruit RNA polymerase. The construction of this protein complex at the transcription start site and the synthesis of the first phosphodiester bond between nucleotides are referred to as *transcription initiation* (3). The RNA polymerase must successfully transcribe an appropriate length of RNA without premature termination *(elongation).* Pre-

mature termination appears to be a regulated mechanism that controls expression of a small number of genes. Transcription of the RNA must also terminate appropriately *(termination).*

Transcription Initiation: A Critical Biological Control Point

As described in the preceding section, transcription can be divided into three discrete steps: initiation, mRNA chain elongation, and chain termination. Although biologically significant regulation may occur at any step in the process, transcription initiation appears to be one of the most significant control points that gates the flow of information out of the genome. Certainly, as far as we know now, transcription initiation is the step in gene expression that is most highly regulated by extracellular signals (3).

Transcription initiation involves two critical processes: positioning of the appropriate RNA polymerase at the correct start sites of transcription and controlling the efficiency of initiations to produce the appropriate transcriptional rate for the circumstances of the cell. These control functions depend on regulatory elements that recruit appropriate transcription factors to the DNA (Fig. 17.1). Many transcription factors bind DNA directly; others interact indirectly through protein–protein interactions with factors that do bind DNA themselves. Those regulatory elements that set the transcription start sites of a gene are called the *basal or core promoters.* Other regulatory elements tether additional activator and repressor proteins to the DNA.

Core Promoters: Setting the Start Site and Direction of Transcription

In eukaryotes, transcription is carried out by three distinct RNA polymerases: RNA polymerases I, II, and III (4). These three polymerases interact with different classes of genes, each of which contains distinct promoter elements. Polymerase I (pol I) promoters are used by genes that encode large rRNAs (ribosomal RNAs). Polymerase II (pol II) promoters are used by genes that are transcribed to yield mRNAs and hence proteins. Pol II promoters are also used by a subset of the genes that encode snRNAs that are involved in RNA splicing. Polymerase III (pol III) promoters are used by genes that encode other small RNAs, including the remaining snRNAs, small rRNAs, and tRNAs (transfer RNAs).

None of the RNA polymerases bind DNA directly; rather, the polymerases are recruited to the DNA by other proteins. The core promoters for each of the three polymerases contain distinct elements on which different types of basal transcription complexes are assembled, each using different transcription factors. Because the main focus of this chapter is regulated expression of protein-encoding genes, only transcription by pol II is described.

The core promoters of genes transcribed by pol II are surprisingly diverse, but they share certain key features. By far the most common core promoter element for pol II promoters is the *TATA box* (Fig. 17.1), a sequence rich in the nucleotides A and T located between 25 and 30 bases upstream of the transcription start site. In TATA box–containing genes, mutation of this sequence can inhibit tran-

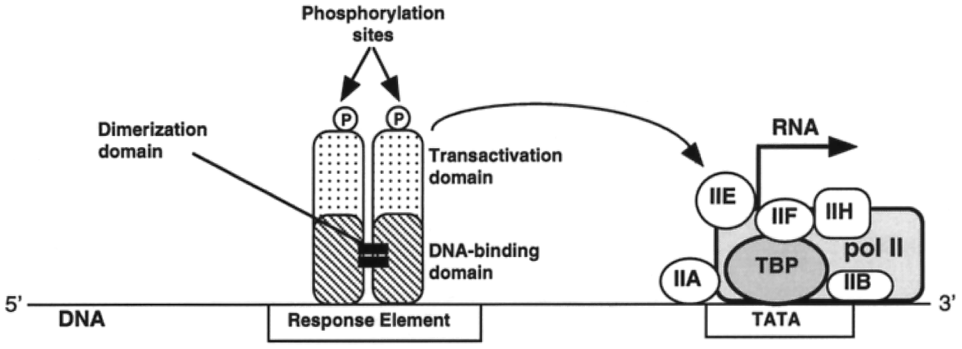

FIGURE 17.1. Scheme of a generalized polymerase II promoter. The figure shows two regulatory elements *(open rectangles)* along the stretch of DNA *(thin black line).* These include the TATA element and a hypothetic activator or response element. The TATA element is shown binding the TATA-binding protein, TBP. Multiple general transcription factors and RNA polymerase II (pol II) associate with TBP. The general transcription factors are referred to with the nomenclature of TFII(x), for transcription factors of a pol II promoter. Shown are general factors, TFIIA, B, E, F, and H. Each of these transcription ''factors'' is actually composed of multiple individual proteins complexed together. TBP and its associated proteins are collectively called TFIID. This basal transcription apparatus recruits RNA polymerase II. It also forms the substrate for interactions with various activator proteins that bind to activator elements such as the one shown. Typical activator proteins contain DNA-binding domains, dimerization domains, and transcription activation domains. The latter interact with the basal transcription apparatus and may be modified by phosphorylation. Adapted from reference 14.

scription initiation or make it inaccurate. In addition to setting the start site of transcription, the TATA box sets the orientation of the basal transcription complex and therefore the 5′ to 3′ direction in which pol II synthesizes the RNA. Many pol II promoters (including those for many neurally expressed genes) lack a TATA box; in these cases, a poorly conserved core promoter element called an *initiator* is found.

The TATA-binding protein (TBP) initiates the formation of the basal transcription complex along with multiple TBP-associated proteins (TAFs) and multiple additional general transcription factors (Fig. 17.1). Each of the transcription factors represented in Fig. 17.1 was originally identified as a chromatographic fraction derived from cell nuclei, and it is a mixture of proteins. Thus, TBP together with its TAFs was originally identified as a fraction called TFIID, where TFII is a nomenclature identifying general transcription factors associated with pol II, and the final letter designates the fraction. TFIID, but not TBP by itself, is required to build a basal transcription complex from TATA-less promoters.

Transcription Factors: Key Regulators of Gene Expression

The basal transcription apparatus is not adequate to initiate more than low levels of transcription. To achieve significant levels of transcription, this multiprotein assembly requires help from additional transcriptional activators that recognize and bind to regulatory elements found elsewhere within the gene. Because they are tethered to DNA—by their binding to specific recognition sequences in the DNA—such proteins can be described as sequence-specific transcription factors (5–7).

Functional regulatory elements are generally found within several hundred bases of the start site of the gene to which they are linked, but they can occasionally be found many thousands of base pairs (bp) away, either upstream or downstream of the start site. Regulatory elements that exert control near the core promoter itself have been called *promoter elements,* and those that act at a distance have been called *enhancer elements,* but the distinction between promoter and enhancer elements is artificial from a mechanistic point of view. Both are generally composed of small, modular elements (generally 7 to 12 bp in length), each of which is a specific binding site for one or more transcription factors. The fundamental logic of transcriptional regulation in eukaryotes is that it is combinatorial: each gene has a particular combination of regulatory elements, the nature, number, and spatial arrangement of which determines the gene's unique pattern of expression. These promoter or enhancer elements control the cell types in which the gene is expressed, the times during development in which it is expressed, and the level at which it is expressed in adults both basally and in response to physiologic and environmental signals (7).

Sequence-specific transcription factors typically contain several physically distinct functional domains (these are shown in Fig. 17.1): (a) the DNA-binding domain recognizes and binds to a specific nucleotide sequence (i.e., response element); (b) the transcription activation domain interacts with coactivators or with general transcription factors (i.e., components of the pol II complex) to form a mature or fully active transcription complex; and (c) the multimerization domain permits the formation of homomultimers and heteromultimers with other transcription factors. The modularity of these proteins is emphasized by the finding that particular binding domains, activation domains, and interaction domains are used in different combinations in many naturally occurring proteins. Experimentally, domains can be swapped from different activators to produce novel hybrid proteins that are functionally active.

Many transcription factors are active only as dimers or higher-order complexes. Multimerization domains are diverse and include so-called leucine zippers (described later), Src homology (SH-2) domains, and certain helical motifs (8–10). Within transcription factor dimers, whether they are homodimers or heterodimers, both partners commonly contribute jointly to both the DNA binding domain and to the activation domain. In some cases, dimerization can be a mechanism of negative control of transcription. This is illustrated by the CREB (cyclic adenosine monophosphate [cAMP]–response element binding protein) family of transcription factors discussed later.

Regulation of transcription factors by the formation of heterodimers is not an "all or none" proposition, however. Within the Fos family of transcription factors (described later), some family members, such as c-Fos, are strong activators when dimerized with a partner from the Jun family, such as c-Jun. Other Fos-related proteins, such as Fra1 (Fos-related antigen–1), bind DNA as heterodimers with c-Jun, and they may still activate transcription, but at lower levels than c-Fos (11). Overall, the ability of transcription factors to form heterodimers and other multimers increases the diversity of transcription factor complexes that can form in cells and, as a result, increases the types of specific regulatory information that can be exerted on gene expression.

Sequence-specific transcriptional activator and repressor proteins may contact several proteins within the basal transcription complex directly. In other cases, they interact with the basal transcription apparatus through the mediation of coactivator or adapter proteins. In either of these situations, transcription factors that bind at a distance from the core promoter can still interact with the basal transcription apparatus, because the DNA forms loops that bring distant regions in contact with each other.

Many activator proteins become involved only in the assembly of the mature transcription apparatus after modification, most commonly phosphorylation, that occurs in re-

sponse to extracellular signals. An important effect of many phosphorylation events is to alter the ability of the phosphoprotein to interact with other proteins. This is illustrated by CREB, which can activate transcription only when phosphorylated on a particular serine residue (ser133) (12). As seen later, phosphorylation of ser133 permits CREB to interact with an adapter protein, CBP (CREB-binding protein), which, in turn, contacts and activates the basal transcription apparatus (13).

REGULATION OF GENE EXPRESSION BY EXTRACELLULAR SIGNALS

Transcription Factors: Targets of Signaling Pathways

Most genes probably contain response elements that confer responsiveness to physiologic signals. Response elements work by binding transcription factors that are activated (or inhibited) by specific physiologic signals, of which the most common is *phosphorylation.* Two general mechanisms of transcriptional regulation by extracellular signals are illustrated Fig. 17.2 (14). In one mechanism, transcription factors that are present at significant levels in cells under basal conditions are rapidly activated by signaling cascades to activate or repress transcription of responsive target genes. In the other major mechanism, transcription factors that are expressed at very low levels under basal conditions are themselves induced by a physiologic signal, after which they can regulate expression of a series of additional genes.

A critical step in extracellular regulation of gene expression is the transduction of signals from the cell membrane to the nucleus; this can be accomplished by several different types of mechanisms. Some transcription factors themselves translocate to the nucleus on activation. One example is provided by the steroid hormone receptor transcription factors, discussed at length later. Another example is the transcription factor nuclear factor-κB (NF-κB) (15). This transcription factor is retained in the cytoplasm by its binding protein, IκB, which masks the nuclear localization signal within NF-κB. Signal-regulated phosphorylation of IκB by protein kinase C leads to dissociation of NF-κB, which permits it to enter the nucleus, where it can bind DNA; IκB is then proteolyzed within the cytoplasm.

Other transcription factors must be directly phosphorylated or dephosphorylated to bind DNA. For example, phosphorylation of STATs (signal transducers and activators of transcription) by protein tyrosine kinases in the cytoplasm permits their multimerization, which, in turn, permits nuclear translocation and construction of an effective DNA binding site within the multimer (16).

Still other transcription factors are already bound to their cognate *cis*-regulatory elements in the nucleus under basal conditions and are converted into transcriptional activators by phosphorylation. CREB, for example, is bound to DNA elements termed cAMP-response elements (CREs) (Fig. 17.3) before cell stimulation. The critical nuclear translocation step in CREB activation involves not the transcription factor itself, but activated protein kinases (cAMP-dependent protein kinase; also called protein kinase A) that, on entering the nucleus, phosphorylate CREB. Alternatively, CREB activation can involve the nuclear translocation of second messengers, such as Ca^{2+} bound to calmodulin, which, on entering the nucleus activate protein kinases that then phosphorylate CREB (Fig. 17.3). As stated earlier, phosphorylation converts CREB into a transcriptional activator by permitting it to recruit CBP into the transcription complex.

The remainder of this chapter provides a more in-depth discussion of several transcription factor families that have received a great deal of attention as mediators of neural and behavioral plasticity in the adult.

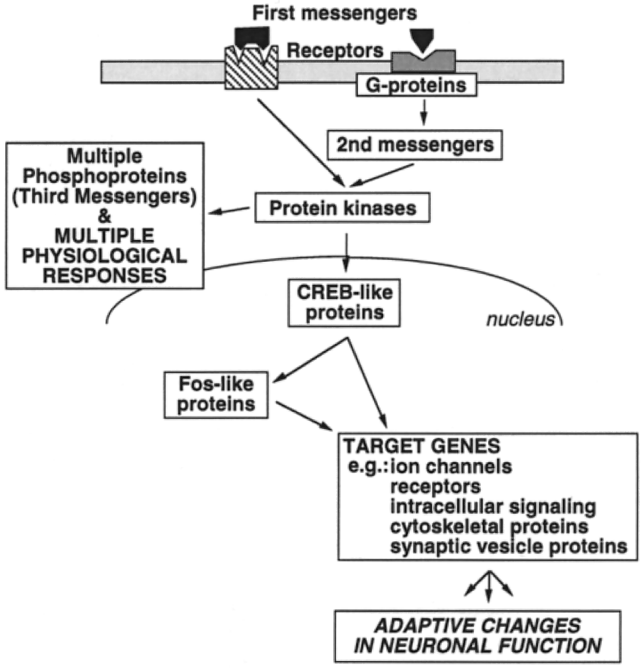

FIGURE 17.2. Scheme of intracellular pathways underlying regulation of gene expression. Activation of neurotransmitter, hormone, or neurotrophic factor receptors leads to the activation of specific second messenger and protein phosphorylation pathways, which produce multiple effects on neuronal function through the phosphorylation of numerous proteins. Among the effects of these intracellular pathways on neuronal function is the regulation of gene expression. This can be accomplished by two basic types of mechanisms. In one case, transcription factors, already in the nucleus, are phosphorylated by protein kinases; this alters their transcriptional activity and leads to alterations in the expression of specific target genes. CREB is an example of a transcription factor that functions in this manner. Among the target genes for CREB family transcription factors are those for other transcription factors, for example, Fos and Jun family proteins. Increased expression of Fos and Jun then leads to alterations in the expression of additional target genes.

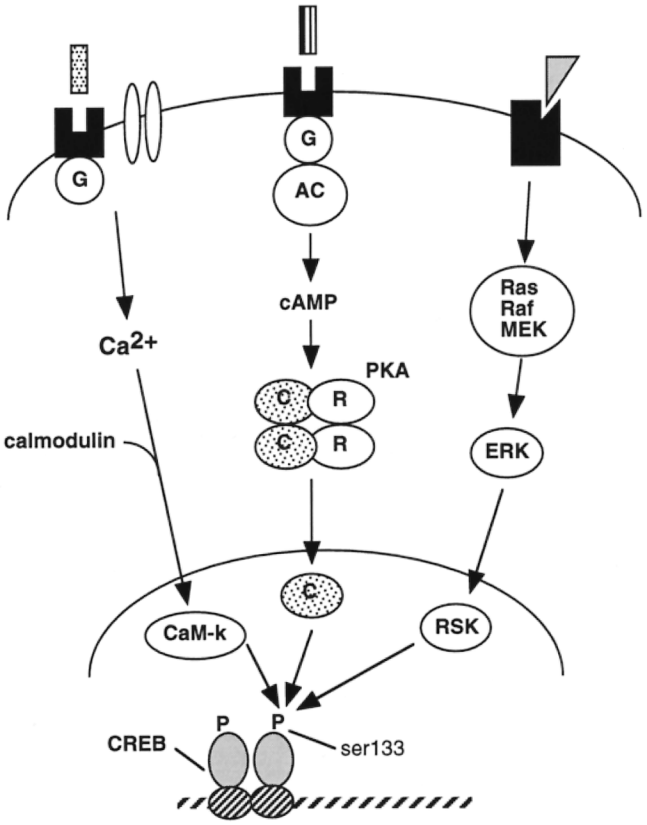

FIGURE 17.3. Scheme of the regulation of CREB phosphorylation by several signaling pathways. The figure illustrates how several signaling pathways converge on the phosphorylation of CREB at a single serine residue, ser133. Neurotransmitters that stimulate adenylyl cyclase would increase CREB phosphorylation by the activation of protein kinase A (PKA). On activation, free PKA catalytic subunits would translocate to the nucleus, where they would phosphorylate ser133 of CREB. Neurotransmitters that inhibit adenylyl cyclase would cause the opposite cascade and inhibit CREB phosphorylation. Any of several signals that increase cellular Ca^{2+} levels (e.g., certain inotropic or G-protein–coupled receptors, voltage-gated Ca^{2+} channels) would also increase CREB phosphorylation. Here, it appears that a wave of increased Ca^{2+} would permeate the nucleus, where it would activate certain Ca^{2+}/calmodulin–dependent protein kinases (CaM kinases), particularly CaM-K IV, which phosphorylates ser133 of CREB. In addition, growth factor regulated pathways lead to CREB phosphorylation, although the details are not as well established. One possibility, shown in the figure, is that activation of Ras-Raf-MEK pathways would lead to activation of ERK (a type of MAP kinase), which would translocate to the nucleus and phosphorylate and activate RSK (ribosomal S6 kinase). RSK would then phosphorylate ser133 of CREB. MEK, MAP kinase and ERK kinase; ERK, extracellular signal regulated kinase; RSK, ribosomal S6 kinase.

CREB Family of Transcription Factors

CREs were the first second messenger response element to be well characterized (12,17,18). As the name suggests, CREs confer activation by cAMP on genes to which it is linked. Subsequently, it was found that the same element confers response to Ca^{2+} and to the Ras pathway as well. CREs have been identified in many genes expressed in the nervous system, including those encoding neuropeptides (e.g., somatostatin, proenkephalin, vasoactive intestinal polypeptide), neurotransmitter synthetic enzymes (e.g., tyrosine hydroxylase), signaling proteins (e.g., adenylyl cyclase type VIII), and transcription factors (e.g., c-Fos and CREB itself) (12,17,18).

The idealized or "consensus" CRE sequence is TGACGTCA, although the actual CREs present in various genes differ slightly. The consensus CRE sequence illustrates an important principle, the palindromic nature of many transcription factor–binding sites. Examining the sequence TGACGTCA, it can be readily observed that the sequence on the two complementary DNA strands, which run in opposite directions, is identical. Many regulatory elements are perfect or approximate palindromes because many transcription factors bind DNA as dimers, with each member of the dimer recognizing one of the half-sites. The major protein that binds to CREs is CREB. CREB binds to a CRE as a homodimer, with a higher affinity for palindromic than for asymmetric CREs.

When bound to a CRE, CREB activates transcription only when it is phosphorylated on its critical ser133. It does so, as described earlier, because phosphorylated CREB, but not dephosphorylated CREB, can recruit the adapter protein, CBP, into the transcription complex. CBP, in turn, interacts with the basal transcription complex.

Regulation by cAMP, Ca^{2+}, and Growth Factors

As discussed in previous sections, the regulation of CREB activation by phosphorylation illustrates the requirement for nuclear translocation of protein kinases or second messengers when transcription factors are already found in the nucleus under basal conditions and the role of phosphorylation in regulating protein–protein interactions. An additional important principle illustrated by CREB is the convergence of signaling pathways. CREB is activated in response to activation of the cAMP or Ca^{2+} pathways. This occurs because the same serine residue (ser133) is phosphorylated both by protein kinase A and by Ca^{2+}/calmodulin–dependent protein kinases (CaM kinases) (Fig. 17.3). CaM kinase IV appears to be the most important form of the enzyme that mediates this phosphorylation (19,20). CREB also appears to be phosphorylated on ser133 by a growth factor–activated kinase, RSK—ribosomal S6 kinase—that is phosphorylated and activated by mitogen-activated protein (MAP) kinases (21).

Thus, diverse types of signaling pathways converge on the phosphorylation and activation of CREB. If each individual signal is relatively weak, convergence may be a critical mechanism for achieving significant gene regulation, with some genes activated only when multiple pathways are stimulated. Furthermore, some genes that contain CREs are known to be induced in a synergistic fashion by the interaction of cAMP and Ca^{2+} signaling pathways. In addition

to ser133, CREB contains other sites for phosphorylation by a variety of protein kinases, which may fine tune the regulation of CREB-mediated transcription. For example, CaM kinase II phosphorylates a distinct serine residue in CREB, which diminishes the ability of other kinases to phosphorylate ser133. Activation of CaM kinase II would therefore appear to mediate a dampening of the CREB signal (19,20).

Role in Neural Plasticity

The convergent activation of a single transcription factor by multiple signaling pathways is particularly important in the nervous system, because this is an important candidate mechanism for long-term neural adaptations, including those underlying long-term memory, drug addiction, and fear conditioning. As discussed elsewhere in this volume, it is reasonably well established that some forms of long-term memory require new gene expression. Furthermore, associative memory depends on the temporally coordinated arrival of two different signals, which must then be integrated within target neurons and their circuits. Activation of CREB is therefore a plausible candidate for playing an important role in long-term memory and related phenomena. Consistent with this prediction, *Drosophila* in which CREB was inactivated by a dominant negative transgene, and mice in which CREB was inactivated by homologous recombination (i.e., knockout), show deficits in long-term memory (22–25). Manipulation of CREB also influences long-term potentiation in the hippocampus (23–25) and aspects of drug addiction (26–29). Although much work remains to understand the precise role of CREB in these various phenomena, it does appear that CREB, which is regulated by several major neural signaling pathways, is a critical mediator of many types of plasticity.

CREB-like Proteins

CREB illustrates yet another important principle of transcriptional regulation: CREB is a member of a larger family of related proteins. Many transcription factors are members of families. CREB is closely related to proteins called the *activating transcription factors* (ATFs) and the *CRE modulators* (CREMs), each generated by distinct genes. In addition, several alternative splice forms are known for CREB, certain of the ATFs, and CREMs (30,31).

All these proteins bind CREs as dimers, and many can heterodimerize with CREB itself. ATF1 appears to be very similar to CREB; it can be activated by both the cAMP and Ca^{2+} signaling pathways (30,31). Many of the other ATF proteins and CREM isoforms also appear to activate transcription. However, certain CREMs (e.g., ICER—inducible cAMP element repressor) act to repress it (30). These CREM isoforms lack the glutamine-rich transcriptional activation domain found in CREB-ATF family proteins that are transcriptional activators. Thus, CREB-ICER heterodimers may occupy CREs, but fail to activate transcription. Like CREB, many of the ATF proteins are constitutively made in cells, but ATF3 and certain CREM isoforms (e.g., ICER) are inducible.

Leucine Zipper Dimerization Motif

The dimerization domain used by the CREB-ATF proteins and several other families of transcription factors is called a *leucine zipper* (8,9). This domain was first identified in the transcription factor C/EBP (CAATT-enhancing binding protein) (32), and it is also used by the AP-1 proteins, as described in detail later. The so-called leucine zipper actually forms a coiled coil. The dimerization motif is an α helix in which every seventh residue is a leucine; based on the periodicity of α helices, the leucines line up along one face of the α helix two turns apart. The aligned leucines of the two dimerization partners interact hydrophobically to stabilize the dimer. In CREB, C/EBP, and many AP-1 proteins, the leucine zipper is at the C-terminus of the protein. There is a region of highly basic amino acid residues just upstream of the leucine zipper that forms the DNA binding domain. Dimerization by the leucine zipper juxtaposes the adjacent basic regions of each of the partners; these juxtaposed basic regions undergo a conformational change when they bind DNA, which results in what has been described as a ''scissors grip.'' This combination of motifs has led this superfamily of proteins to be described as the basic-leucine zipper proteins or the bZIP proteins.

AP-1 Family of Transcription Factors

Another group of bZIP transcription factors that plays an important role in the regulation of neural gene expression by extracellular signals comprises the activator protein-1 (AP-1) proteins. The name AP-1 was originally applied to a transcriptional activity stimulated by protein kinase C activation (33). This activity was found to be composed of multiple proteins, which bind as heterodimers (and a few as homodimers) to the DNA sequence TGACTCA, the AP-1 sequence. The consensus AP-1 sequence is a heptamer that forms a palindrome flanking a central C or G, and differs from the CRE sequence by only a single base. Yet this one base difference strongly biases protein binding away from CREB (which requires an intact CGTCA motif) to the AP-1 family of proteins and means that, under most circumstances, this sequence will not confer cAMP responsiveness on a gene.

Many genes expressed in the nervous system contain AP-1 sites within their regulatory regions (34–36). Examples include genes encoding neuropeptides (neurotensin and substance P), neurotransmitter receptors (D1 dopamine, NR1 NMDA, and GluR2 AMPA glutamate receptor subunits), neurotransmitter synthetic enzymes (tyrosine hy-

droxylase), and cytoskeletal proteins (neurofilament proteins), to name a few. In some cases, it has been possible to demonstrate a role for these sites in regulation of gene promoter activity *in vitro,* although it has been very difficult to identify with certainty those genes that are regulated by AP-1 transcription factors in the brain *in vivo* (35).

As alluded to earlier, the AP-1 sequence was described initially as a TPA-response element (TRE) because the phorbol ester, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), which activates protein kinase C, can induce gene expression through AP-1 proteins (33). In addition, it is now thought that a major role of the AP-1 sequence is to confer responsiveness to growth factor–stimulated signaling pathways such as the Ras/MAP-kinase pathways. This occurs by phosphorylation of specific AP-1 proteins by certain MAP kinases.

AP-1 proteins generally bind DNA as heterodimers composed of one Fos family member and one Jun family member (34). Both families are bZIP proteins: they form dimers through their leucine zipper domains. The known members of the Fos family are c-Fos, Fra1, Fra2, and FosB and its alternatively spliced variant ΔFosB. The known members of the Jun family are c-Jun, JunB, and JunD. Unlike Fos proteins, c-Jun and JunD (but not JunB) can form homodimers that bind to AP-1 sites, albeit with far lower affinity than Fos-Jun heterodimers. The potential complexity of transcriptional regulation is greater still because some AP-1 proteins can heterodimerize through the leucine zipper with members of the CREB-ATF family, such as ATF2 with c-Jun. AP-1 proteins can also form higher-order complexes with apparently unrelated families of transcription factors. For example, AP-1 proteins can complex with and thereby apparently inhibit the transcriptional activity of steroid hormone receptors (see later).

Among Fos and Jun proteins, only JunD is expressed constitutively at high levels in many cell types. The other AP-1 proteins tend to be expressed at low or even undetectable levels under basal conditions, but, with stimulation, they may be induced to high levels of expression. Thus, unlike regulation by constitutively expressed transcription factors such as CREB, regulation by Fos-Jun heterodimers requires new transcription and translation of the transcription factors themselves (Fig. 17.2).

Cellular Immediate Early Genes

Genes that are transcriptionally activated by synaptic activity, drugs, or growth factors have often been classified roughly into two groups. *Immediate early genes* (IEGs), such as the c-*fos* gene itself, are activated rapidly (within minutes) and transiently and do not require new protein synthesis. *Late-response genes,* in contrast, are induced or repressed more slowly (over hours) and are dependent on new protein synthesis. The term IEG was applied initially to describe viral genes that are activated ''immediately'' on infection of eukaryotic cells, by commandeering host cell transcription factors for their expression. Viral IEGs generally encode transcription factors needed to activate viral ''late'' gene expression. This terminology has been extended to cellular (i.e., nonviral) genes, although this has created some confusion.

The terminology is problematic because there are many cellular genes induced independently of protein synthesis, but with a time course intermediate between ''classic'' IEGs and late-response genes. In fact, some genes may be regulated with different time courses or requirements for protein synthesis in response to different extracellular signals. Moreover, many cellular genes regulated as IEGs encode proteins that are not transcription factors: for example, any gene induced by CREB could potentially show temporal features of induction of an IEG. Despite these caveats, the concept of IEG-encoded transcription factors in the nervous system has proved useful in thinking about the complexities of gene regulation. In addition, because of their rapid induction from low basal levels in response to neuronal depolarization (the critical signal being Ca^{2+} entry) as well as various second messenger and growth factor pathways, several IEGs have been used as cellular markers of neural activation, and this has permitted novel approaches to functional neuroanatomy (37).

The protein products of those cellular IEGs that function as transcription factors bind to regulatory elements contained within a subset of late response genes to activate or repress them. IEGs such as c-fos have therefore been termed ''third messengers'' in signal transduction cascades, with neurotransmitters designated intercellular first messengers and small intracellular molecules, such as cAMP and Ca^{2+}, second messengers (34). There have, however, been misunderstandings among some neurobiologists that IEGs are a necessary step in the signal-induced expression of most neural genes involved in the differentiated function of neurons. In fact, as stated earlier, many genes involved in differentiated neural functions, including genes encoding certain neuropeptides and neurotrophic factors, to name a few, are activated in response to neuronal depolarization or cAMP through phosphorylation of CREB rather than through IEG third messengers.

Activation by Multiple Signaling Pathways

The most studied cellular immediate early gene is c-*fos.* The c-*fos* gene contains three binding sites for CREB (the strongest of which is shown in Fig. 17.4). As a result, it is not surprising that the gene can be activated rapidly by neurotransmitters or drugs that stimulate the cAMP or Ca^{2+} pathways (38).

The c-*fos* gene also can be induced by the Ras/MAP-kinase pathway (39,40). Neurotrophins, such as nerve growth factor, bind a family of *receptor tyrosine kinases* (called Trks) that activate Ras. Ras then triggers a cascade

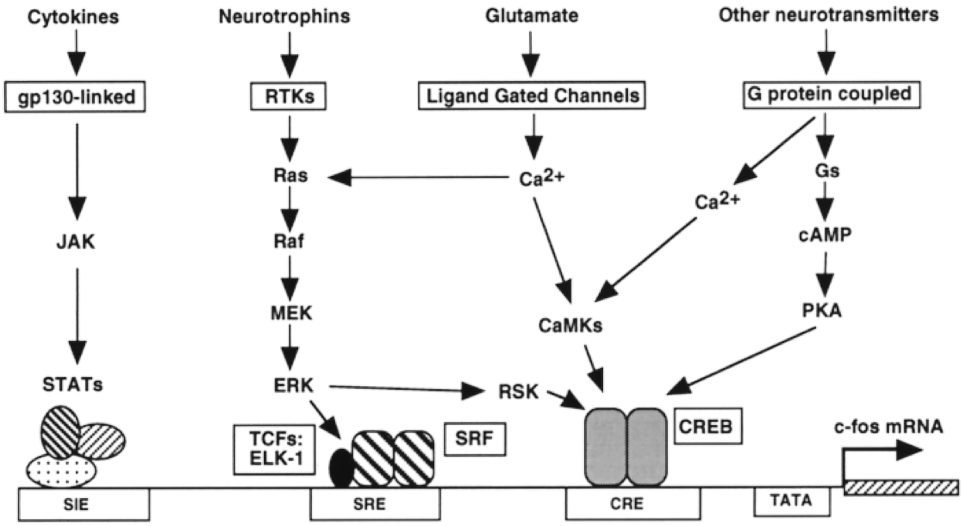

FIGURE 17.4. Scheme of the regulatory region of the c-*fos* gene. The figure shows only three of the many known transcription factor binding sites within this gene. These sites are as follows: a CRE (cAMP-response element), which binds CREB; a serum-response element (SRE), which binds serum response factor (SRF) and Elk-1 (also called the ternary complex factor or TCF); and an SIF-inducible element (SIE), which binds STAT proteins (signal transducers and activators of transcription). Proteins binding at each of these sites are constitutively present in cells and are activated by phosphorylation. CREB can be activated by protein kinase A, CaM kinases (CaM-Ks), or RSKs (ribosomal S6 kinases) (Fig. 17.3); Elk-1 can be activated by the MAP kinases ERK1 and ERK2 (extracellular signal regulated kinases 1 and 2); and the STAT proteins can be activated by the JAK protein tyrosine kinases. Thus, activation of the c-*fos* gene—by any of multiple signaling pathways—depends only on signal-induced phosphorylation rather than on new protein synthesis. This explains the rapidity of its induction by a wide array of stimuli. MEK, MAP kinase and ERK kinase; PKA, protein kinase A; RTKs, receptor tyrosine kinases. Adapted from reference 14.

of protein kinases, which results in the phosphorylation and activation of certain MAP kinases called *extracellular signal regulated kinases* (ERKs). These ERKs can phosphorylate and activate additional protein kinases, such as RSK, which, among its other substrates, can phosphorylate ser133 of CREB, as described earlier. However, an additional mechanism exists whereby ERK can induce the c-*fos* gene, and this mechanism appears to predominate in many cell types (41). Here, the ERKs translocate into the nucleus where they phosphorylate the transcription factor Elk-1 (also called the ternary complex factor or TCF). Elk-1 then complexes with the serum response factor (SRF) to bind to and activate the serum response element (SRE) within the c-*fos* gene (Fig. 17.4). SREs are present within many other growth factor-inducible genes as well. In comparison with cAMP- or Ca^{2+}-dependent phosphorylation of CREB, the Ras/MAP kinase pathway depends on a complex chain of phosphorylation events. Nonetheless, these events can occur very rapidly to induce gene expression.

Still another mechanism exists for c-*fos* induction: cytokine-activated signaling pathways that act through STATs (42). As stated earlier, STATs are activated on their phosphorylation by certain protein tyrosine kinases. This permits STATs to form multimeric complexes, translocate to the nucleus, and bind to their specific DNA response elements, generally now described at STAT sites. However, the STAT site in c-*fos* had already been named the SIE or SIF-inducible element (SIF itself is an acronym for sis-inducible factor, i.e., a factor induced by the oncogene v-*sis,* which activated c-*fos* though this site). STATs are activated by the class of cytokines that interact with gp130-linked receptors, which include ciliary neurotrophic factor, LIF (leukemia inhibitory factor), interleukin-6, leptin, and prolactin, to name a few (16,43). These receptors activate a cytoplasmic protein tyrosine kinase called JAK (Janus kinase), which then phosphorylates the STATs. As shown in Fig. 17.4, the c-*fos* gene contains an SIE, which binds STAT proteins and mediates the induction of c-Fos by cytokines.

Most other Fos and Jun family proteins are also induced rapidly in response to diverse acute stimuli and, presumably, many of the same mechanisms operate for the genes encoding these proteins. However, the response elements within these genes are not as well characterized as are those for c-*fos,* and further research is needed to understand their regulation.

Regulation by Phosphorylation

Several AP-1 proteins are regulated at the post-translational level by phosphorylation. The best-established example is

the phosphorylation of c-Jun, which provides a critical mechanism of regulation of AP-1-mediated signaling (44, 45). c-Jun phosphorylation occurs in response to activation of a MAP kinase–related signaling pathway that is activated by many forms of cellular stress. In this pathway, a Ras-like G protein is activated by any of several insults (e.g., ultraviolet radiation, osmotic stress, toxins, certain cytokines); this triggers a cascade of protein kinases analogous to that triggered by Ras and the neurotrophins outlined earlier. The culmination of this pathway is the phosphorylation and activation of certain MAP kinases called SAP kinases (stress-activated protein kinases) or alternatively JNKs (for Jun N-terminal kinases). JNKs phosphorylate c-Jun on serines 63 and 73 in its transcriptional activation domain and increase the ability of c-Jun to activate transcription. Phosphorylation and activation of c-Jun have been implicated in the regulation of apoptosis (programmed cell death) pathways (45).

Generation of Unique AP-1 Complexes by Repeated Stimulation

After acute stimulation of cells, different members of the Fos family are induced with varying time courses of expression, which leads to a progression of distinct AP-1 complexes over time (46). Thus, under resting conditions, c-*fos* mRNA and protein are barely detectable in most neurons, but c-*fos* expression can be induced dramatically in response to numerous stimuli. As just one example, experimental induction of a grand mal seizure causes marked increases in levels of c-*fos* mRNA in brain within 30 minutes and induces substantial levels of c-Fos protein within 2 hours. c-Fos is highly unstable and is degraded back to low, basal levels within 4 to 6 hours (46). Administration of cocaine or amphetamine causes a similar pattern of c-*fos* expression in striatum (47,48). In either of these stimulus paradigms, other Fos-like proteins are also induced, but with a longer temporal latency than c-Fos; their peak levels of expression lag behind c-Fos by approximately 1 to 2 hours. Moreover, expression of these proteins persists a bit longer than c-Fos, but it still returns to basal levels within 8 to 12 hours.

With repeated stimulation, however, the c-*fos* gene, and to an extent the genes for other Fos-like proteins, become refractory to further activation (i.e., their expression becomes desensitized) (49,50). Instead, other Fos-like proteins continue to be expressed. These proteins, originally termed chronic Fras (51–53), are now known to be biochemically modified isoforms of ΔFosB, which exhibit very long half-lives in brain (54–56). As a result, these proteins accumulate in specific neurons in response to repeated perturbations and persist long after cessation of these perturbations (Fig. 17.5). Although the precise physiologic significance of these stable ΔFosB isoforms remains unknown, there is now direct evidence that ΔFosB plays an important role in aspects of drug addiction (57) and in mediating various types of striatal-based movement disorders (e.g., see refs. 58–60). More generally, ΔFosB may function as a sustained molecular switch that gradually converts an acute response to a long-lived adaptation in the brain (61).

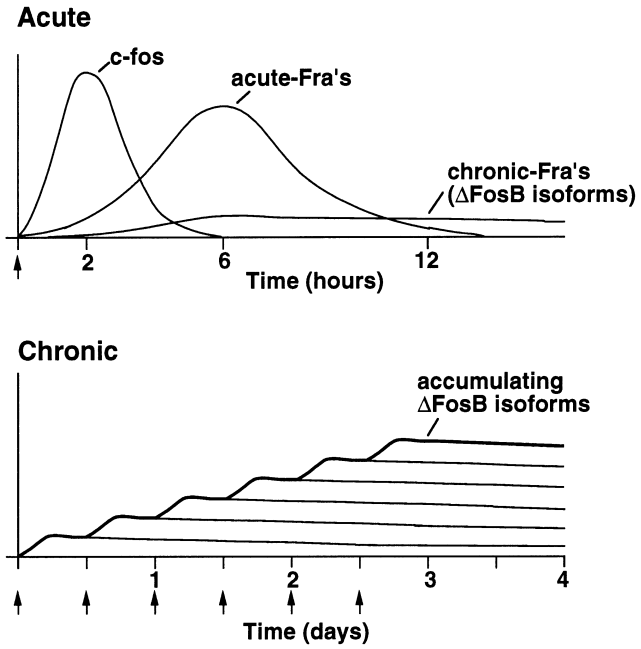

FIGURE 17.5. Scheme showing the composition of AP-1 complexes changing over time. **Top:** There are several waves of Fras (Fos-related antigens) induced by many acute stimuli in neurons. C-Fos is induced rapidly and degraded within several hours of the acute stimulus, whereas other ''acute Fras'' (e.g., FosB, ΔFosB, Fra1, Fra2) are induced somewhat later and persist somewhat longer than c-Fos. The ''chronic Fras'' are biochemically modified isoforms of ΔFosB; they, too, are induced (although at low levels) after a single acute stimulus but persist in brain because of their enhanced stability. In complexes with Jun family proteins, these waves of Fras form AP-1 binding complexes with shifting composition over time. **Bottom:** With repeated stimulation, each acute stimulus induces a low level of ΔFosB isoforms. This is indicated by the lower set of overlapping lines, which indicate ΔFosB-induced by each acute stimulus. The result is a gradual increase in the total levels of ΔFosB with repeated stimuli during a course of long-term treatment. This is indicated by the increasing *stepped line* in the graph. The increasing levels of ΔFosB with repeated stimulation would result in the gradual induction of significant levels of a long-lasting AP-1 complex, which could underlie persisting forms of neural plasticity in the brain. (Adapted from reference 53.)

Steroid Hormone Receptor (or Nuclear Receptor) Superfamily

The steroid hormones (e.g., glucocorticoids, gonadal steroids such as estrogen and testosterone, and mineralocorticoids), retinoids, thyroid hormones, and vitamin D_3 are small, lipid-soluble ligands that diffuse readily across cell

membranes. Unlike amino acid–derived neurotransmitters and neuropeptides, their receptors are cytoplasmic, rather than localized to the cell membrane. On ligand binding, these receptors translocate to the nucleus, whereupon they bind to specific hormone-response elements (HREs) located in the regulatory regions of specific genes and thereby regulate expression of those genes. These receptors are referred to as the *steroid hormone receptor, or nuclear receptor, superfamily* (62–64).

The glucocorticoid receptor (GR) exemplifies general mechanisms utilized by this superfamily (62–65). Under basal conditions, the GR is retained in the cytoplasm by a large multiprotein complex of chaperone proteins, including the heat shock protein Hsp90 and the immunophilin Hsp56. When bound by glucocorticoid, the GR dissociates from its chaperones and translocates to the nucleus. The first activity of the GR to be identified was its function as a ligand-regulated transcription factor, as stated earlier, by binding to glucocorticoid response elements (GREs). GREs are typically 15 bases in length; they consist of two palindromic half-sites of six bases each separated by a 3-bp spacer. As described earlier for other transcription factors, this palindromic organization of the GRE suggests that the GR binds as a dimer. Like many other transcription factors, the nuclear receptor superfamily has a modular structure. The GR has three domains: an N-terminal transcriptional activation domain, a C-terminal ligand binding domain, and an intervening DNA binding domain. The DNA-binding domain of the GR is characterized by a zinc finger motif, in which multiple cysteines are organized around a central zinc ion. This type of DNA binding domain is used by many other transcription factors, including the immediate early gene zif268/egr1 (see later). The DNA binding domain also contains a region that permits dimer formation after GR monomers bind GRE half-sites.

GREs can confer either positive or negative regulation on genes to which they are linked, depending on the particular GRE involved (62–65). One of the first positive GREs characterized is that within the metallothionein IIA gene, which encodes a protein that chelates heavy metals. A well-characterized negative GRE is found within the proopiomelanocortin *(POMC)* gene. This negative GRE permits glucocorticoids to repress the gene that encodes adrenocorticotropic hormone and is therefore an important mechanism of feedback inhibition on further glucocorticoid synthesis.

GRs have many important physiologic actions that do not appear to be mediated by DNA binding. GRs can interfere with transcriptional activity mediated by other transcription factors, particularly AP-1 and NF-κB. Although the mechanisms are not fully understood, GRs appear to interact directly with AP-1 and NF-κB proteins to block their ability to activate transcription (62–65). An alternative mechanism by which glucocorticoids may interfere with NF-κB activity is by inducing expression of IκB, the protein that holds NF-κB in the cytoplasm.

Other Transcription Factors in Neural Signaling

The CREB, AP-1, STATs, and steroid hormone receptor families are just a few of the literally hundreds, perhaps thousands, of transcription factors that are expressed in neurons and glia. Most of these other factors are best understood with respect to their roles in nonnervous tissues, although more recent work implicates them in neural signaling as well. Examples of these other transcription factors have already been mentioned. NF-κB is activated by protein kinase C and immunologic signals and likely plays an important role in the regulation of neural gene expression (15). C/EBP and its several family members are known to mediate some of the effects of the cAMP pathway on gene expression and have been implicated in neural plasticity (32, 66). Specific protein-1 (SP-1), which binds to GC rich regions of promoters, is often thought of as a general transcription factor, that is, a regulator of the basal rate of transcription. However, more recent research has shown that certain SP-1 family members are subject to dynamic regulation and could mediate transcriptional changes induced by extracellular signals (5). AP-2 (activator protein-2) binding sites are present in many neural-expressed genes, although we still know very little about its physiologic role (5). Zif268 (also called Egr-1) and related Egr family members are zinc finger transcription factors that, like c-Fos, are induced rapidly and transiently in brain by many stimuli with temporal features of IEGs. Induction of Egr family proteins has been correlated with induction of hippocampal long-term potentiation; however, their specific target genes remain poorly characterized (67).

CONCLUSIONS

Our discussion of nuclear signaling mechanisms highlights several important points. The first is that the potential number of mechanisms by which the expression of a gene can be controlled is vast. This highlights the exquisite control over gene expression that is required both for the generation of a diversity of cell types during development and for adaptation of cells to the environment throughout life.

We devoted most attention to nuclear transcription factors, because these provide the best-understood mechanisms of how cells adapt to external cues with alterations in gene expression. However, even the complexity of mechanisms discussed represents the tip of the iceberg. Regulatory regions of genes are often far longer than the coding regions of genes. Regulatory information is contained not only within the 5′ promoter regions of genes, but throughout intronic (and sometimes exonic) sequences as well as 3′ untranscribed regions. Within the 5′ regions, we focused on relatively small response elements, such as CRE and AP-1 sites. It is extraordinary, indeed, that the difference of 1

nucleotide (e.g., from a CRE to an AP-1 site) in a sequence of literally thousands can confer specificity on a gene with respect to its regulation. Nonetheless, we know that any given gene likely contains many regulatory sites. Moreover, these sites do not function in isolation, but they influence one another. As a result, the rate of expression of a given gene represents the temporal and spatial synthesis of multiple signaling pathways. Unraveling these layers of complexity is a daunting task, particularly *in vivo,* but it could hold important clues for understanding neural and behavioral plasticity.

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